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July 31, 2017

Dr. Shaunta Hill-Hammond
Biologist and Designated Federal Officer
U.S. EPA: Office of the Administrator, Science Advisory Board Staff Office
1200 Pennsylvania Avenue, NW (1400R)
Washington, DC 20460-0001
Hill-Hammond.Shaunta@epa.gov

Re: EPA Draft Toxicological Review of *tert*- Butyl Alcohol (*tert*-Butanol)(CAS No. 75-65-0) In Support of Summary Information on the Integrated Risk Information System (IRIS) June 2017. EPA/635/R-16/015a

Dear Dr. Hill-Hammond:

LyondellBasell appreciates the opportunity to provide comments to the U.S. Environmental Protection Agency (EPA) and the SAB Chemical Assessment Advisory Committee augmented for the review of the EPA draft assessment: Toxicological Review of *tert*-Butyl Alcohol (*tert*-Butanol)(CAS No. 75-65-0) in response to the June 16, 2017 Federal Register notice [FRL-9963-24-OA] announcing the availability of the External Review Draft for its Toxicological Review of *tert*- Butyl Alcohol (TBA), which was prepared to support the summary information that appears on EPA's Integrated Risk Information System (IRIS). LyondellBasell, as a producer of TBA, has a significant interest in any review of TBA's health and environmental risks by an authoritative body. We are committed to accurate and up-to-date scientific information and assessments of our products and hence we have reviewed and provide comments on the TBA External Review Draft assessment to assure that the latest information is available so that the best possible assessment is achieved.

The present External Review Draft of the TBA IRIS Assessment is similar to the April 2016 External Peer Review Draft, hence many of our comments submitted previously to the EPA on July 13, 2016 are still valid and should be considered for the present External Review Draft. As with the previous version, the External Review Draft's assessment of the TBA health effects database is not supported by the available scientific information and draws some erroneous conclusions regarding TBA's potential for human health hazards and risks.

Our main issues with the External Draft Review for TBA are summarized below:

- There is very strong evidence that TBA is not genotoxic. Yet the External Review Draft concludes that no definitive conclusion can be made and thus the potential genotoxicity effects of TBA cannot be discounted. This evaluation reflects inexperience with genotoxicity data that undermines the scientific credibility of the External Review Draft

assessment. We provide comments from Dr. Bhaskar Gollapudi, an internationally recognized expert in the field of genetic toxicology.

- The EPA's conclusion that kidney tumors in male rats are relevant to human hazard identification is not scientifically supported. Contrary to the External Review Draft's conclusion, no "unknown processes" are involved in renal tumor formation. There is very strong evidence that weak effects of an α-2u-globulin mode of action (MOA) are working additively, and in concert with very advanced CPN to produce a weak renal tubule tumor response. These are the only two MOA's operating for the renal tumors, and they are not relevant to humans. The overall weight of evidence suggests that the mode of action for thyroid tumors is most likely anti-thyroid. This MOA is not relevant to humans.
- The External Review Draft does not adequately discuss uncertainties regarding the excessively high dose(s) used in the mouse thyroid tumor assessment, which exceed EPA and OECD guidance addressing the limitations of toxicity responses observed at dose levels saturating metabolic saturation with resulting nonlinear toxicokinetics of TBA.
- The oral slope factor should be clearly annotated with the conclusion that the overall "suggestive evidence" of TBA carcinogenicity does not allow for its use in quantitative human risk analyses. It would be more appropriate to provide both a threshold and non-threshold estimate for thyroid tumors to capture the uncertainty of the risk assessment.
- The oral reference dose is not scientifically supported because it is based on increases in severity of nephropathy in females that are completely explained by changes involving chronic progressive nephropathy. This MOA does not occur in humans and should not be used to derive an oral reference dose.
- The External Review Draft's analysis incorrectly teases apart and analyzes each type of histopathology kidney lesion as if separate independent features, which is antithetical to the manner in which knowledgeable pathologists would recognize the separate lesions as part of the single disease process and record it as such. This means that dose response assessment based on any one component lesion is highly inaccurate because the pathologist is not always recording these lesions individually if the pathologist records CPN to represent the constellation of related findings.
- The External Review Draft assessment and EPA's response to previous comments by LyondellBasell on the 2016 preliminary draft assessment reflect a lack of pathology expertise and understanding of CPN. We provide comments from Dr. Gordon Hard, who led the Pathology Working Group microscopic evaluation of the kidney.

When the TBA assessment considers these points, it will find:

1) TBA is not a cancer concern for humans.

2) TBA is a low concern to humans for chronic toxicity by inhalation and oral exposure.

Our conclusions are supported in our attached comments:

- LyondellBasell Comments on Charge Questions to the Science Advisory Board for the IRIS Toxicological Review of *tert*- Butyl Alcohol. June 2017. Submitted July 31, 2017.

We urge the EPA and the augmented SAB Chemical Assessment Advisory Committee to review these and our detailed previous comments on the April 2016 External Peer Review Draft submitted to the EPA on July 13, 2016 and available in the EPA docket EPA-HQ-ORD-2013-0111-0021 (https://www.regulations.gov/document?D=EPA-HQ-ORD-2013-0111-0021)

LyondellBasell appreciates your consideration of our comments.

Regards,

LyondellBasell

Attachment

LyondellBasell Comments on Charge Question to the Science Advisory Board for the IRIS Toxicological Review of *tert*-Butanol June 2017

July 31, 2017

1. Literature Search Strategy/ Study Selection and Evaluation- Systematic Review Methods

Please comment on the strategies for literature searches, criteria for study inclusion or exclusion, and evaluations of study methods and quality discussed in the Literature Search Strategy/ Study Selection and Evaluation section. Were the strategies clearly described and objectively applied?

LyondellBasell Comment:

Study Selection:

In view of commonly identified central nervous system (CNS) effects associated with butanol isomers, the External Review Draft should include discussion of available neurotoxicity and developmental neurotoxicity studies of butanol isomers in the assessment of *tert*-Butanol (TBA). For example, inhalation neurotoxicity studies on isobutanol include functional observational battery, motor activity, schedule-controlled operant behavior and perfusion fixation neuropathology (Li et al. 1999).

The External Review Draft's rationale for excluding TBA studies solely on the basis that these studies evaluated physical dependency is not appropriate. If the study design is similar to a repeat dose toxicology study with neurobehavioral or other appropriate toxicological measures before and after a withdrawal period, then this study design is similar to a toxicology study with a "recovery period".

The External Review Draft summarized the toxicity of chemicals extensively metabolized to TBA (e.g. ETBE and MTBE) for some non-cancer and cancer (e.g. Sections 1.1.4, 1.3.1 and 1.3.2), but then indicated in response to our previous 2016 comments that for neurotoxicity the "assessment is focused on the toxicity resulting from TBA exposure as the parent compound" (p. D-11 line 4). Although the toxicological effects may not be due solely to TBA, the absence of findings at time points when only TBA is found in blood can provide additional supportive read-across data that is useful for hazard characterization for TBA. Ethyl tertiary butyl ether (ETBE) and methyl tertiary butyl ether (MTBE) studies are informative for read-across hazard characterization of the neurotoxicity effects of TBA, especially if blood levels of TBA from these compounds are available.

Evaluations of study methods and quality:

The Preamble and page xxxii of the External Review Draft clearly indicate that the study methods and quality including statistical methods are taken into consideration when evaluating

the overall contribution to the synthesis of evidence. However, the External Review Draft's stated approach for evaluation was not always objective for critical endpoints:

- The evaluation of the genotoxicity studies lacked critical evaluation of the quality of the studies that should have given much greater weight to a robust battery of negative genotoxicity studies conducted by NTP rather than conclude that there was inadequate data to make a conclusion based on few positive studies that were weaker or flawed studies. Detailed comments on genotoxicity are provided below in our response to Charge Question 4a.

- Similarly, in the evaluation of the mode of action (MOA) for chronic progressive nephropathy (CPN), the External Review Draft gives evaluation of the kidney lesions by a pathology working group (Hard et al. 2011, 2013) equal weight as an evaluation by Melnick et al. (2012, 2013). Hard et al. (2011) reviewed the microscopic slides of the TBA kidneys blind. Melnick et al. (2013) is an editorial commentary that was not peer-reviewed. Melnick et al. (2012) did not evaluate the TBA kidney slides and conducted analysis that was flawed as described below in our response to Charge Question 4.

- Study methods and quality of the Acharya et al. (1997) paper were not taken into consideration in the External Review Draft's evaluation of the kidney lesions (see detailed response to Charge Question 3a). Because of poor quality in reporting of histopathology, the study of Acharya et al. (1997) is not a reliable source of information regarding the relevance of TBA to human health considerations. The histologic description strongly suggests the kidney tissues in this study were affected by post mortem changes, and therefore this paper/study would not be a reliable source of information.

Hazard Identification and Dose-Response Analysis

2. Chemical Properties and Toxicokinetics

2a. Chemical properties. Is the information on chemical properties accurate?

<u>LyondellBasell Comment</u>:

The information on TBA's chemical properties appears to be accurate.

2b. Toxicokinetic modeling. Section B.1.5 of Appendix B in the Supplemental Information describes the application and modification of a physiologically-based toxicokinetic model of *tert*-butanol in rats (Borghoff et al., 2016). Is use of the model appropriate and clearly described, including assumptions and uncertainties? Are there additional peer-reviewed studies that should be considered for modeling?

LyondellBasell Comment:

The Supplemental Information, Section B.1.5 of Appendix B, includes reference to two ETBE-TBA PBPK models (Salazar et al., 2015 and Borghoff et al., 2016) and a review of these models by the EPA Pharmacokinetics Working Group (US EPA, 2017). Although noted that the Borghoff et al. (2016) model was used for the route-to-route extrapolation, it is not clear that the modifications identified in the US EPA 2017 review were incorporated. As such when the question above is asked, is the use of the model appropriate and clearly described, including assumptions and uncertainties, the model version and parameter values that were used and this version of the model and its limitations should be identified. Also, it is not clear in the US EPA, 2017 assessment of these models that the modifications to the Borghoff et al. 2016 model demonstrate an overall better fit to the available TBA tissue data following varying exposure scenarios and if model uncertainties might have influenced the route to route extrapolation used for estimating the oral slope factor.

As noted on page B-8 "For simulating exposure to drinking water, the water consumption was modeled as episodic, based on the pattern of drinking observed in rats (Spiteri, 1982). In particular, rats were assumed to ingest water in pulses or "bouts" which were treated as periods of continuous ingestion, interspersed with periods of no ingestion. During the active dark period (12 hours/day), it is assumed that 80% of total daily ingestion occurs in 45-minute bouts alternating with 45 minutes of other activity. During the relatively inactive light period (12 hours/day), it is assumed that the remaining 20% of daily ingestion occurs; the bouts are only assumed to last 30 minutes, with 2.5 hours between. This pattern is thought to be more realistic than assuming continuous 24 hours/day ingestion. The resulting ingestion rate for one exposure is shown in Figure B-2.

It is assumed, but not clearly stated, that this description was incorporated into the Borghoff et al. 2016 model and used for the route-to-route extrapolation for calculating an inhalation reference concentration for noncancer outcomes. In the Borghoff et al. 2016 model, the drinking water exposure was estimated as a daily dose (mg/kg bw/day) of ETBE or TBA using the animal's body weights to calculate a constant infusion rate (mg/hour) into the gut lumen over a 12-hour simulation period. Although it is stated that the pulses or bouts over a 12-hour period is more realistic, there was no data to support that this was better at predicting TBA tissue levels vs. the original description in the Borghoff et al. 2016 model. As such, any uncertainty with respect to the description of water consumption described in the model should be identified.

US EPA, 2017, Page 5, line 30
In the review of the ETBE-TBA PBPK models (US EPA, 2017) the following statements were made "Binding to α-2u-globulin is one hypothesized mode of action for the observed kidney effects of MTBE-exposed animals. In the Leavens and Borghoff (2009) model, binding of MTBE to α-2u-globulin was applied to describe sex differences in kidney concentration of MTBE and tert-butanol, but acceptable estimates of MTBE and tert-butanol pharmacokinetics in the blood are predicted in other models that did not consider α-2u-globulin." These statements are true. However, a critical point is that a description of binding of MTBE and or TBA to α-2u-globulin α-2u-globulin along with decreased rate of α-2u-globulin protein catabolism in the

kidney due to binding is needed to predict the concentrations of MTBE and TBA in the male rat kidney following exposure. Hence, a model would not predict the kidney concentration of either MTBE or TBA, the site in which responses are observed, without a description of chemical binding to this protein. Chemical binding to α-2u-globulin does not change the concentration of a chemical in the blood, but changes the concentration in the kidney since it slows the catabolism of the bound protein resulting in a slower clearance of the chemical from the kidney. This point is critical for predicting the clearance of TBA from the male rat kidney and the need to describe TBA binding to α-2u-globulin in the male rat kidney. It is important in consideration of a dose metric especially for responses in the target tissue, as is the case of TBA.

Some of the features of the Borghoff et al. 2016 model were further clarified in this assessment (US EPA, 2017) and a list of suggested changes provided. However, it was never quite clear if these changes, when implemented in the Borghoff et al. 2016 model, would lead to better predictions of TBA tissue data. In the US EPA 2017 model assessment document, it was not always clear what model or model version was ultimately used for route-to-route extrapolation for calculating an inhalation reference concentration.

The final question "Are there additional peer-reviewed studies that should be considered for modeling?" It appears that all the peer-reviewed studies along with toxicokinetic study reports have been used in the development of the most updated model version (Borghoff et al., 2016) and considered in this assessment. However, now that the definitive ETBE and TBA elimination study is incorporated (Borghoff and Asgharian, 1996) it is not clear why the pilot studies by Sun and Beskitt (1995a,b) and Borghoff (1996) are still used.

2c. Choice of dose metric. Is the average concentration of *tert*-butanol in blood an appropriate choice for the dose metric?

<u>LyondellBasell Comment:</u>

Considering that the route-to route extrapolation was carried out using the average concentration of TBA in blood as the dose-metric, it is critical that the reasoning for selection of this dose metric is clearly justified in the main review and supported in the supplemental information.

Since the External Review Draft considered the non-cancer effect to be based on nephrotoxicity in the female kidney, and since TBA does not bind to α-2u-globulin in the female kidney, the concentration of TBA in female rat blood would reflect the concentration of TBA in the female rat kidney. As such, it may be the most appropriate dose metric for route to route extrapolation to derive an RfC, that is, if the nephrotoxicity in the female rat is the endpoint selected. We do not support that the RfC be derived based on female rat kidney nephrotoxicity since it is CPN which is identified as not relevant to humans. With this being the case, a dose metric is not needed for a quantitative assessment based on endpoints identified in the rat.

Hazard Identification and Dose–Response Assessment

3. Noncancer

3a. Noncancer kidney toxicity (Sections 1.2.1, 1.3.1). The draft assessment identifies kidney effects as a potential human hazard of *tert*-butanol. EPA evaluated the evidence, including the role of α-2u-globulin and chronic progressive nephropathy, in accordance with EPA guidance (U.S. EPA, 1991). Please comment on whether this conclusion is scientifically supported and clearly described.

LyondelBasell Comment:

The conclusion of a Pathology Working Group (PWG) is that there is overwhelming evidence that weak effects of an α-2u-globulin MOA are working additively, and in concert with, the weak effect of very advanced CPN to produce a very weak renal tubule tumor response (Hard et al. 2011, 2013). Neither of these MOA is relevant to humans, and the histopathology evidence does not support a conclusion that there are other unknown MOA.

The External Review Draft analysis incorrectly does not consider CPN as a diagnostic entity, when in fact this is consistent with recommendations of the various Societies of Toxicologic Pathology (Frazier et al. 2012). The External Review Draft's analysis incorrectly teases apart and analyzes each type of histopathology lesion as if separate independent features, which is antithetical to the manner in which knowledgeable pathologists would recognize the separate lesions as part of the single disease process and record it as such. Specifically, when CPN is recorded as a single diagnostic entity, the component lesions of cell death/degeneration (cytotoxicity), basophilic tubule, regenerative tubule, hyaline cast, are not recorded separately. The External Review Draft gives equal weight to both Melnick et al. (2012, 2013) and Hard et al. (2011) papers despite major flaws with the analysis by Melnick et al. that have been discussed in detail by Hard et al. (2013) and Dr. Sam Cohen's public comments.

The External Review Draft's responses to Cohen, Hard and LyondellBasell comments (p. D-4, line 7) rationalizes that the correlation between renal tumors and CPN is weak in female rats, and therefore the renal tumors (in males) cannot be attributed solely to CPN. This is not scientifically valid because it does not account for the α-2u-globulin nephropathy MOA in males and highlights an apparent lack of understanding of CPN. The External Review Draft's response to LyondellBasell comment that hyaline droplet accumulation was not given adequate importance and more discussion of the hyaline droplet pathology should be included was ignored because "additional text describing hyaline droplets would not affect overall conclusion of the MOA nor would it serve to increase the clarity of the decision." This response seems to imply that the External Review Draft approach to the α-2u-globulin MOA was inadequate and biased.

As discussed in the following detailed comments, the External Review Draft assessment and EPA's response to previous comments by LyondellBasell on the 2016 preliminary draft assessment reflect a lack of pathology expertise and understanding of CPN.

Detailed Comments on Charge Question 3a from Dr. Gordon Hard, an internationally recognized renal toxicologic pathologist:

The External Review Draft maintains the position that certain kidney effects associated with the test article are relevant for human hazard identification, and that unknown processes contribute to renal tumor development after TBA exposure (page 1-67, lines 1-2). This is a weak and speculative conclusion unsupported by any suggested possibilities.

Despite arguing that the data often is inconsistent or insufficient (page 1-30), this document acknowledges that the kidney tumors resulted in part from a MOA involving α-2u-globulin MOA nephropathy that is specific to male rats (page 1-39, lines 34-35; page 1-67, lines 27-28). The EPA also acknowledge that "CPN might be involved in the induction of renal tubule tumors in male rats, likely by providing proliferative stimulus in the form of compensatory regeneration following *toxicity* to the renal tubule epithelium". The statement that CPN contributes to the renal tumor formation is supported by some of the data, but there is no evidence to link this spontaneous disease process to toxicity (further comments on CPN and toxicity, below).

It should be noted here that proliferative activity can be observed in all phases of CPN progression, from the earliest development (as seen at 6-7 weeks of age) up to and including advanced CPN. Mitotic activity in CPN-affected tubules is an inherent feature of CPN. It can be regarded as compensatory regenerative response to the etiological agent or process that causes continuing injury to the renal tubules, but it does not represent compensatory regeneration to test article-induced toxic insult. It would be most enlightening if the exact cause of CPN was known, but in the absence of that knowledge, it is not correct to disregard the robust information that is known, including its consistent morphology, progressive nature, and proven link to renal tubule tumor development in the most advanced stages of severity (Hard et al, 2012).

There is no evidence that "some other, yet unspecified, processes" are involved. In the NTP chronic study of TBA, there was no evidence of cytotoxicity in renal tubule cells that were not involved in the CPN process, arguing against a toxic effect of the chemical (Hard, 2005; 2011)[1].There seems to be reluctance by the External Review Draft to accept that the weak effects of an α-2u-globulin MOA could work additively, and in concert with, the weak effect of very advanced CPN to produce a very weak renal tubule tumor response (a non-significant increase in kidney tumors in male rats after standard single section evaluation).

The External Review Draft argues that certain non-cancer kidney effects observed in female rats

[1] Based on re-examination of kidney sections (both standard and extended evaluation step sections) from male rats that had an NTP diagnosis of either adenoma, carcinoma or marked renal tubule hyperplasia, including those from the 15 month sacrifice.

exposed to TBA "are suitable for consideration for dose-response analysis and derivation of reference values". These statements are also used in the decision that some other, unspecified, process is contributing to the adverse kidney effects associated with TBA. Most relevant here is the External Review Draft repeated denial that CPN is a specific diagnosis, and instead, that the individual component lesions of CPN should be considered separately if CPN is exacerbated. Experience with thousands of cases of CPN in the rat, including chemically induced exacerbation, supports without doubt the specific nature and consistent morphology of CPN in the rat, and therefore, the validity of acknowledging it as a single diagnostic entity.

Kidney effects in male rats were not considered in dose-response analysis because these were "complicated" by α-2u-globulin. The specific end-points that were chosen in female rats for this analysis were absolute kidney weights, suppurative inflammation, transitional epithelial hyperplasia, and increases in severity of CPN. In direct contrast to the document's statements, at least 3 of these 4 non-cancer effects in female rats exposed to TBA *are* related to CPN.

Suppurative inflammation is often observed in the rat as occasional foci within advanced CPN. This lesion consists of tubule lumen aggregates of degenerating polymorphonuclear neutrophil leucocytes (PMNs), where the PMNs are responding to bacterial contamination. The most important point regarding this lesion is that in the rat kidney, PMNs are a hallmark of bacterial infection, usually arising via ascending invasion from the lower urinary tract. In this setting of CPN, suppurative inflammation does *not* represent toxicity. It is also very possible that, in different studies, there might be gender differences in incidence of this CPN-associated lesion.

The External Review Draft response to these comments regarding suppurative inflammation implies that our comments were accepted, and clarification was added to the Executive Summary. However, in the Executive Summary (and in the body of the revised document) they continue to argue that suppurative inflammation is a toxic response to TBA and relevant to humans. In the specific case of TBA, the suppurative inflammation was part of the advanced CPN spectrum as acknowledged by NTP in their report, TR346 "Inflammation of the kidneys also regarded as part of the nephropathy…..".[2] . Also, the External Review Draft's responses to the comments do not appear to acknowledge that bacterial involvement can be identified without actually seeing bacteria. As described in the previous paragraph, bacterial involvement in inflammatory foci in kidney is characterized by the presence of PMNs, and PMN aggregates are the feature identifying foci of suppurative inflammation in the TBA study.

The External Review Draft states that "*Additional histopathology changes including tubular degeneration … were noted in a 10-week study in male rats (Acharya et al, 1997)" (*p. 1-8, lines 19-22; p. 1-12, Table 1-2, and p. 1-28, lines 4-6). Because of poor quality in reporting of histopathology, the study of Acharya et al. (1997) is not a reliable source of information regarding the relevance of TBA to human health considerations. Their observation of "degeneration of renal tubules with syncytial arrangements of the nucleus of renal tubule epithelial cells" in TBA exposed Wistar rats does not accord with accepted histopathology

[2] NTP referred to this "inflammation" as suppurative inflammation" when tabulating the renal observations in female rats in Table 8

nomenclature (Frazier et al., 2012), and suggests the tissues may have been affected by early autolytic change. Despite previous comments from LyondellBasell concerning this data source of insufficient quality, the External Review Draft has continued to use this study in the 2017 assessment of TBA. The histologic description strongly suggests the kidney tissues in this study were affected by post mortem changes, and therefore this study is a doubtful source of reliable information.

Transitional cell hyperplasia has been discussed under ETBE, but this lesion is a papillary lining response to advanced stages of CPN and should definitely not be used to indicate test article-induced nephrotoxic alteration. Teasing it out from the CPN spectrum and suggesting it may individually signal potential human kidney injury is invalid and inappropriate. This specific lesion does not appear to occur in humans.

CPN was exacerbated in both male and female rats, a common side effect of a number of chemicals. In the External Review Draft, EPA emphasizes that there were no renal tumors in the female rats despite the presence of "relatively high-grade" CPN (page 1-36, lines 26-28). EPA is incorrectly assuming that increased severity of CPN of 2.9 or more on a 1-4 scale will necessarily lead to renal tumors. Using a grading system of 1-4, the NTP data show grades of 3.3 for high-dose males versus grade 2.9 for high-dose females, but Hard et al. (2011) found 19 female rats surviving 700 or more days of the NTP study had an average CPN severity grade of 3.2, while CPN in high-dose tumor-bearing males had a higher average CPN grade of 3.5.However, Hard et al. (2012, 2013) emphasize that the association between advanced CPN and renal tumor development is very subtle, and that a 1 to 4 grading system is not sufficiently sensitive to show a definite correlation between advanced CPN and renal tubule tumor development. This is precisely the reason for applying a 0 to 8 severity grading system in an experimental approach for identifying this link. It is also one of the reasons why the Melnick et al. (2012) partial review of the NTP database failed to show a positive trend for this association. Hard et al. (2012) have shown that CPN-associated renal tubule adenoma development in untreated, control male or female rats mainly occurs in animals with end-stage (grade 8) CPN. Furthermore, not all rats with grade 8 (end-stage) CPN would be expected to develop a renal tumor. In the Hard et al. (2012) review of CPN, which histopathologically re-examined kidneys from more than twenty 2-year NTP rat carcinogenicity studies, the incidence of low-grade renal tubule tumors occurring in control male rats with end-stage (grade 8) CPN was only 10.6%. The absence of renal tumors in the TBA-exposed high-dose female group is consistent with long-standing experience of the CPN disease process in female rats.

The External Review Draft uses exacerbation of CPN as an indicator of nephrotoxicity by including it in the list of kidney effects that they consider relevant for human hazard assessment and for derivation of reference values (page 1-65, lines 12-16; Page 2-2, lines 11-13). Exacerbation of CPN, although caused by various chemicals, represents enhancement of a spontaneous disease process, which is controlled by multiple dietary and hormonal factors. As such, it should not be regarded as a nephrotoxic event. There is no evidence to link it to nephrotoxicity, and the External Review Draft approach is purely speculative. It should be re-emphasized here that the document's repeated statement that "CPN is not a specific diagnosis" is incorrect. CPN is a specific disease process occurring in laboratory rodents, especially the rat,

and is acknowledged as such by the international Societies of Toxicologic Pathology (Frazier et al., 2012).

Increases in kidney weights are non-specific and can be caused by changes unrelated to nephrotoxicity. They should not be used as an indicator of renal toxicity in this analysis as increases in kidney weights in the TBA studies are most likely due to exacerbated CPN, and exacerbated CPN is not a nephrotoxic response.

3b. Noncancer toxicity at other sites. (Sections 1.2.3-6, and 1.3.1). The draft assessment finds inadequate information to assess developmental, neurodevelopmental, and reproductive toxicity. Please comment on whether these conclusions are scientifically supported and clearly described. If there are publicly available studies to associate other health outcomes with *tert*- butanol exposure, please identify them and outline the rationale for including them in the assessment.

LyondellBasell Comment:

The data to assess developmental, neurodevelopmental, and reproductive toxicity for TBA are limited. The External Review Draft appropriately included information on ETBE and MTBE reproductive toxicity studies. In response to previous LyondellBasell comments that there are relevant neurotoxicity studies on butanol isomers, EPA stated in Appendix D that EPA is focused on studies on the parent, yet EPA added new data on MTBE and ETBE for hazard characterization of other endpoints. Studies on MTBE and ETBE can be informative for hazard characterization of the effects of TBA, especially if blood levels of TBA from these compounds are available. In addition, there are studies on neurotoxicity of TBA and other structural related chemicals.

Developmental Toxicity (Section 1.2.3)

The External Review Draft conclusion has been changed regarding TBA developmental toxicity hazard to include the conclusion that it is not possible to determine if the developmental toxicity observed occurred due to direct effects of TBA exposure or if the developmental toxicity observed was secondary to maternal toxicity. The previous 2016 draft had concluded that the developmental toxicity evidence for TBA was “suggestive” of an effect and that the maternal toxicity observed was “minimal”. The External Review Draft corrects both of those misconceptions.

While the current External Review Draft describes the maternal toxicity in somewhat greater detail than the May 2016 version, it still fails to acknowledge significant shortcomings in the detail and presentation of the maternal effects. For example, maternal body weight loss in the Nelson et al. (1989) study are still described as reductions in body weight gain while body weight loss is much more severe an outcome than a reduction in body weight gain. Examination of the Nelson et al. (1989) paper provides data demonstrating (Figure 3 of the paper) a clear negative weight gain (body weight loss: up to 25-30 grams) for the two highest exposure concentrations during the first five days of exposure. The highest exposure concentration did not

return to their gestation day (GD) 0 body weight until GD 14. Negative weight gain means the animals lost body mass during this time and is markedly different that a “decrease in body weight gain”. Furthermore, the unsteady gait was reported at all of the exposure concentrations and this fact is not mentioned in the abbreviated description provided.

In addition, the maternal toxicity observed in the Nelson et al. (1989) study are still not included within the exposure-response array figures for that study and therefore it is impossible for a reader of this document to view the maternal effects along with the developmental effects in a single figure. The maternal effects in other developmental toxicity studies are included within there their respective exposure-response array figures (allowing the side-by-side comparison), this is not done for the Nelson et al. (1989) study and there is no logical reason for this oversight.

An additional example is the notation of the ataxia, lethargy and unresponsiveness within the 1000 mg/kg bw/day dose group dams within the Lyondell (2004) study. However, there is no discussion of how these effects would be expected to affect maternal care of the newborn pups and subsequent effects on postnatal survival and growth of the offspring. Rat pups are altricial when born and require maternal care and manipulation for temperature control, access to nipples for nursing and even to urinate requires maternal stimulation. Dams that are experiencing ataxia, lethargy or are unresponsive (intoxicated) cannot provide these necessary interactions and services to their offspring. Lack of maternal care is an important variable in postnatal survival in rat species.

In addition, there is no examination or consideration of the dose levels used in several of the studies presented as causing developmental toxicity. Several of the cited studies used dose levels many multiples above the limit dose level of 1000 mg/kg bw/day described within the EPA’s own developmental toxicity test guidelines. Developmental toxicity that occurs at these very high dose levels are of minimal relevance to human health and the focus of the draft assessment should be on the studies using lower dose levels/exposure concentrations (e.g. Nelson et al., 1989; Huntingdon Life Sciences, 2004).

Finally, there is no discussion whatsoever of the dose levels used in the developmental toxicity studies and how useful this information is within the IRIS assessment process. The logic and reasoning underpinning the limit dose of 1000 mg/kg bw/day within the US EPA Test Guideline for developmental toxicity testing should be recognized and used by the IRIS group when evaluating studies. For example, both the Faulkner et al. (1989) and Daniel and Evans (1982) publications use dose levels well above the limit dose prescribed within the EPA Test Guideline. Only the Huntingdon Life Sciences (2004) and Nelson et al. (1989) use dose levels/exposure concentrations appropriate under the EPA test Guideline. Nelson et al. (1989) recognized the very high oral dose levels (approximately 3200 mg/kg bw/day) needed to cause developmental toxicity and stated that comparable exposure concentrations of 30,000 ppm would be required to achieve comparable internal dose levels (page 478 of Nelson et al. (1989)).

The External Review Draft should also acknowledge that an increase in resorption frequency is not only due to possible developmental effects but also due to maternal toxicity. Maternal factors and physiological changes are required for the maintenance of pregnancy and allow for normal

intrauterine development. Maternal toxicity endpoints that affect either the maintenance of pregnancy or intrauterine development can also cause an increase in resorptions. Therefore, an increase in resorption frequency should be viewed as an indication of both maternal and developmental toxicity. Currently, the External Review Draft only interprets this endpoint a developmental toxicity without considering maternal factors and the influence of toxicity to the dam on this endpoint.

For example, Faulkner et al. (1989) stated "*Significantly more of the resorptions in the treated groups (33/75) required ammonium sulfide for visualization than in the control group (2/14) (X^2=4.66, p.<0.05), suggesting an early effect on fetal viability*"

The use of the term "fetal viability" within the publication is incorrect as early or embryonic resorptions are, by definition, pregnancy losses that occur during the embryonic period. Ammonium sulfide reacts with the blood remaining in the placental/uterine remnant remaining after an early resorption, allowing visualization of a previous implant that otherwise would be invisible for viewing. There were no other indications of maternal toxicity presented in the study publication, despite using oral dose levels known to cause central nervous system effects in rodents.

The question then becomes, is the early pregnancy loss (resorption) due to maternal toxicity or due to a direct effect on the embryo? This question is impossible to answer with confidence as early resorptions can be due to problems within the conceptus or from lack of maternal factors conducive to development or a combination of both. Without knowing the exact mechanism-of-action leading to these resorptions, a conservative approach would be to list these effects as indications of both maternal and developmental toxicity.

<u>Neurodevelopment (Section 1.2.4)</u>

The External Review Draft is incorrect in concluding that the data provide inadequate information to draw any conclusions regarding neurodevelopmental toxicity of TBA. The data indicate there is no consistent behavioral pattern of developmental neurotoxicity in offspring exposed during gestation to inhalation exposures of approximately (6000 or 12,000 mg/m^3) (Nelson et al., 1991). These exposure levels are more than 10-fold higher than the point of departure of 500 mg/m3 based on route-to-route extrapolation from the RfD of 0.4 mg/kg/day based on female nephropathy.

The Nelson et al. (1991) study is a comprehensive developmental neurotoxicity study that incorporated multiple tests of motor activity, motor coordination, and cognitive behavior including schedule controlled operant behavior. The methods section of the Nelson et al. (1991) publication refers to previous publications for further details that External Review Draft should include in their evaluation of this study. Evaluation of these and other papers by the same authors increases confidence that this laboratory was experienced in conducting the same battery of behavioral tests following inhalation exposures to several alcohols.

The External Review Draft review of this study suggests that the study is limited by the fact that the low and high dose exposures were not run concurrently. This would be similar to running two separate limit dose tests at high exposure levels and finding minimal effects. Nelson et al. (1991) clearly acknowledges this and correctly analyzes the data by comparing the different dose levels with the concurrent control. Despite these limitations, the data provide adequate information to conclude that there is no consistent pattern of developmental neurobehavioral toxicity in offspring exposed during gestation to inhalation exposures of approximately (6000 or 12,000 mg/m^3) (Nelson et al., 1991).

The Nelson et al. (1991) study was much stronger than the Daniel and Evans (1982) study. The External Review Draft needs to indicate in the text (p. 1-55) that Daniel and Evans (1982) did not analyze data with litter as the experimental unit and limited the evaluations to much fewer neurobehavioral endpoints conducted prior to weaning. Although the External Review Draft correctly indicates that Daniel and Evans (1982) did not statistically compare TBA treated animals with control, the External Review Draft should indicate that the numerical values of the lowest dose level of 3324 mg/kg-d (estimated by EPA) are nearly identical to control values (see Table below). The lowest dose level for both studies exceeds the recommended limit dose for developmental and reproduction studies of 1000 mg/kg bw/day by oral administration and 2000 mg/m^3 by inhalation.

Taken together, the data from these two studies indicate that there is no consistent pattern of developmental neurobehavioral toxicity observed at very high dose levels. These observations further support the External Review Draft Section 2.1.3 on database uncertainty factor selection that concludes further studies are unlikely to lead to identification of a more sensitive endpoint or a lower point of departure than the 500 mg/m^3 selected in the External Review Draft for inhalation exposures.

<u>Reproductive Endpoints (Section 1.2.5)</u>

Within our previous review of the May 2016 draft EPA assessment, we stated "The Reproductive Effects Section (1.2.5) of the IRIS document summarizes the available information correctly and the interpretation and conclusion is reasonable and justified."

The only substantial change to this section within the External Review Draft is the introduction of the ETBE one-generation and two generation reproductive toxicity study information as providing further support for the lack of evidence for reproductive effects for TBA. This addition to the External Review Draft is warranted and justified.

3c. Oral reference dose for noncancer kidney outcomes. Section 2.1 presents an oral reference dose of 4x10^{-1} mg/kg^{-day}, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or how the approach might be developed.

LyondellBasell Comment:

The oral reference dose is not scientifically supported because it is based on increases in severity of nephropathy in females that are completely explained by changes involving CPN. This MOA does not occur in humans and should not be used to derive an oral reference dose (Hard et al. 2009, 2011, 2013).

The External Review Draft's dose response assessment based on one of several closely related component lesions for CPN ignores best practices for pathology assessment in a manner that severely undermines the scientific credibility of the External Review Draft. The External Review Draft analysis incorrectly does not consider CPN as a diagnostic entity, when in fact this is how the various Societies of Toxicologic Pathology are recommending kidneys be evaluated (Frazier et al. 2012). The External Review Draft's analysis incorrectly teases apart and analyzes each type of histopathology lesion as if separate independent features, which is antithetical to the manner in which knowledgeable pathologists would recognize the separate lesions as part of the single disease process and record it as such. Specifically, when CPN is recorded as a single diagnostic entity, the component lesions of cell death/degeneration (cytotoxicity), basophilic tubule, regenerative tubule, hyaline cast, are not always recorded separately. This means that dose response assessment based on any one component lesion is highly inaccurate because the pathologist is not recording these lesions individually if the pathologist records CPN to represent the constellation of related findings.

3d. Inhalation reference concentration for noncancer outcomes. Section 2.2 presents an inhalation reference concentration of 5 mg/m^3, based on increases in severity of nephropathy in female rats via drinking water (NTP, 1995), converted for inhalation exposure using a toxicokinetic model (Borghoff et al., 2016). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative data set or approach would be more appropriate, please outline how such data might be used or the approach might be developed.

LyondellBasell Comment:

As described in detail in comments to Question 3a and 3c, the inhalation reference dose is not scientifically supported because it is based on increases in severity of nephropathy in females that are completely explained by CPN. This MOA does not occur in humans and should not be used to derive an inhalation reference dose (Hard et al. 2009, 2011, 2013).

4. Cancer

4a (i) Cancer modes-of-action in the kidney. **As described in section 1.2.1, kidney tumors were observed in male rats following *tert*-butanol exposure, and a mode-of-action involving α-2u-globulin and/or chronic progressive nephropathy was evaluated. The analysis, conducted in accordance with EPA's guidance on renal toxicity and neoplasia in the male rat (U.S. EPA, 1991), considered the kidney tumors in male rats to be relevant to human hazard identification. Please comment on whether this conclusion is scientifically**

supported.

LyondellBasell Comment:

This charge question is confusing because it suggests that the External Review Draft considers the kidney tumors relevant for human hazard identification, yet the External Review Draft did not use the data for cancer risk assessment because of evidence that α-2u-globulin and/or CPN are involved. As discussed in greater detail in response to question 3a, LyondellBasell disagrees that the kidney tumors in male rats are relevant to human hazard identification, but agree with the External Review Draft that the kidney tumors are NOT relevant for derivation of slope factors.

The External Review Draft of the IRIS review of TBA maintains the position that certain kidney effects associated with the test article are relevant for human hazard identification, and that unknown processes contribute to renal tumor development after TBA exposure (page 1-67, lines 1-2). As discussed in greater detail above under question 3a and 3c, there is strong evidence that weak effects of an α-2u-globulin MOA are working additively, and in concert with very advanced CPN to produce a weak renal tubule tumor response. Contrary to the External Review Draft's conclusion, no "unknown processes" are involved in renal tumor formation based on absence of findings in renal tubules unaffected by CPN. These are the only two MOAs that are operating for the renal tumors. The External Review Draft's analysis incorrectly teases apart the histopathology evaluation into separate independent sub-categories, which are all manifestations of CPN, with the addition that the increased kidney weight could also be contributed by the α-2u-globulin nephropathy based on the conclusions of the PWG (Hard et al., 2011).

4a ii. Cancer modes-of-action in the thyroid. **As described in section 1.2.2, thyroid tumors were observed in male and female mice following *tert*-butanol exposure, and an anti-thyroid mode-of-action was evaluated. The analysis, conducted in accordance with EPA's guidance on thyroid follicular cell tumors in rodents (U.S. EPA, 1998), found the information inadequate to determine whether an anti-thyroid mode-of-action was operating and considered the thyroid follicular cell tumors in male and female mice to be relevant to humans. Please comment on whether this conclusion is scientifically supported.**

LyondellBasell Comment**:**

Although the evidence is inadequate to definitively prove an anti-thyroid MOA based on strict (check-box) adherence to EPA's guidance (Hill et al. 1998), the overall weight of evidence suggests that the MOA is most likely anti-thyroid. This MOA is not relevant to humans. Section 1.2.2 of the draft report ignores important dose-response, toxicokinetic and statistical considerations raised in Lyondell's comments to previous drafts of EPA's assessment. The External Review Draft should integrate evidence both in support of and contradictory to the External Review Draft's conclusions into section 1.2.2 so that all the evidence is presented. EPA (2005) cancer risk assessment guidelines emphasize that "proper characterization of uncertainty is essential in risk assessment". EPA can better reflect the uncertainty of their conclusions by providing a non-linear threshold BMDL estimate for the mouse thyroid hyperplasia in addition to

the slope factor for adenoma and carcinoma. This will allow EPA to quantitatively bound the estimates in a manner that better reflects the uncertainty of the External Review Draft assessment.

DETAILED COMMENTS ON SECTION 1.2.2:

There is toxicokinetic data indicating that the top male/female doses of greater than 2000 mg/kg bw/day, and possibly even the female/male mid-doses of 1020 and 1040 mg/kg bw/day, exceeded saturation of TBA metabolism resulting in onset of nonlinear plasma TBA toxicokinetics. EPA should include this data in the assessment of thyroid toxicity in section 1.2.2.

The metabolism of TBA suggests that it is reasonable to predict potential high-dose specific metabolic saturation. As reviewed in McGregor (2010), and likewise summarized in the draft assessment, the primary metabolism of TBA in both rodents and humans is mediated through cytochrome P450 oxidation of the TBA methyl group, resulting in formation of 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate. Cytochrome P450 metabolic oxidation is commonly associated with vulnerability to high-dose metabolic saturation. TBA is not a substrate for alcohol dehydrogenase, and lesser amounts of TBA metabolism proceed through direct glucuronidation of the parent molecule.

Mice (C56BL6) administered single intraperitoneal doses of TBA at doses of 5, 10 and 20 mmol/kg bw (370, 741 and 1482 mg/kg bw) resulted in respective AUC values of 28, 96 and 324 mmol.hrs/L (Faulkner and Hussain, 1989). Thus, a 4-fold increase in dose (5 to 20 mmol/kg bw) resulted in an 11.6-fold increase in systemic AUC; metabolic saturation may have been present even at the next lowest dose of 10 mmol/kg bw in which a 2-fold increase in dose (5 to 10 mmol/kg bw) resulted in a 3.4-fold increase in AUC.

Dose (ip) (mg/kg)	Fold increase (Dose)	TBA AUC (mmol*hr/L)	Fold increase (AUC)
370	1X	28	1X
741	2X	96	**3.4X**
1482	4X	324	**11.6X**

From: Faulkner & Hussain, 1989; bolded values indicative of non-linear pharmacokinetics.

Importantly, the top intraperitoneal (ip) dose of 1482 mg/kg bw used in this study was substantially lower than the top bioassay of 2110 mg/kg bw/day in female mice exhibiting thyroid adenomas in the NTP bioassay. These data indicate that the high-dose specific thyroid adenomas occurred under conditions of non-linear toxicokinetic behavior due to saturated oxidative metabolism. Due to the rapid and essentially complete oral absorption of TBA, the systemic toxicokinetics following intraperitoneal administration are likely to reasonably parallel that of oral absorption.

The External Review Draft's response to these comments is that saturation of TBA is reasonable

and the EPA's PBPK model for rats accounts for this saturation (P. D-16, line 26-36). Although the PBPK model is for rats, as noted by the EPA, the Km used for TBA in the rat model was 0.379 mM which is in the range of the Km's (0.56 to 0.92 mM) reported for mice by Faulkner and Hussain (1989). However, the External Review Draft's response does not address the main point of these comments, which is that these data provide evidence that the high dose and possibly the mid dose of the NTP mouse bioassay most likely exceeded toxicokinetic (metabolic) saturation.

The External Review Draft does not include discussion of selection of the high dose level and toxicokinetic considerations in the evaluation of thyroid tumors. A basic tenet of toxicology is the dose makes the poison. Thus, it is essential that Sections 1.2.2 include toxicokinetic and dose level considerations as part of hazard characterization. Otherwise, the uncertainties of EPA's cancer risk assessment are not transparent.

As discussed above, the data from Faulkner and Hussain (1989) suggest that the high dose level used in the mouse bioassay is above the dose threshold for onset of nonlinear toxicokinetics and 2-fold higher than what EPA (1998) considers to be a limit dose for chronic bioassay. This information should be included in the External Review Draft because it provides important context for evaluating the thyroid tumors. Several comprehensive reviews have emphasized that rodent carcinogenicity responses restricted to high test doses may have questionable human health hazard and risk relevance (Foran et al., 1997; Slikker et al., 2004a,b; Barton et al., 2006; Carmichael et al., 2006; Doe et al., 2006). High-dose specific saturation of metabolic processes, including toxicokinetics, may result in transition to MOAs unique to those at high dose levels and/or represent non-dose proportional augmentation of modes of action operating at lower animal doses. If the onset on nonlinear toxicokinetics is well separated from human exposures, effects noted above the onset nonlinear toxicokinetics are not regarded as quantitatively relevant to humans (Saghir et al. 2012; Bus, 2017; OECD, 2012).

Consistent with the expert reviews, the EPA Cancer Risk Assessment Guidelines (2005) have emphasized that changes in toxicokinetics with increasing dose may result "…*in important differences between high and low dose levels in disposition of the agent or generation of its active forms. These studies play an important role in providing a rationale for dose selection in carcinogenicity studies.*" Similarly OECD (2012) Guidance on Conduct and Design of Carcinogenicity Assays emphasizes that *"Available toxicokinetic data (ADME) should always be taken into account when selecting dose levels for a chronic toxicity or carcinogenicity study. . . Many toxicokinetic processes influencing absorption, distribution, elimination and metabolic activation or detoxication may become saturated at higher doses, resulting in systemic exposures to parent compound or metabolites that would not be expected in the real life human exposures for which risk assessments are needed. . . There is broad acceptance that the top dose should ideally provide some signs of toxicity such as slight depression of body weight gain (not more than 10%), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumours. Excessive toxicity at the top dose level (or any other dose level) may compromise the usefulness of the study and/or quality of data generated. . .Criteria that have evolved for the selection of an adequate top dose*

*level include: (**in particular**) toxicokinetics; saturation of absorption; results of previous repeated dose toxicity studies.*

The main point of LyondellBasell's comment is that it is mainstream scientific thinking that is well accepted by U.S. and international OECD regulatory bodies that onset of dose-dependent toxicokinetic nonlinearities is an essential consideration in the interpretation of cancer tumors. The External Review Draft response appears to miss this main point because no change was made to the External Review Draft assessment. Instead, the External Review Draft's comments focus on justifying NTP's selection of the high dose level for the TBA mouse cancer bioassay based on (a) distinction between "need not" in the EPA test guidelines is different from "shall not" exceed the limit dose of 1000 mg/kg; (b) assertion that EPA guidelines were taken out of context and other sections of the guidance indicate studies above 1000 mg/kg could be useful[3]; (c) suggestion that exceeding EPA's recommended limit dose is common practice based on the number of studies NTP has conducted above 1000 mg/kg-d ; and (d) historical perspective that the NTP TBA studies were conducted 15 years prior to development of the kinetically derived maximum (KMD) dose selection strategy (Saghir et al., 2012), although the External Review Draft acknowledges that these approaches "may provide better study designs in the future" (p. D17 line 6). We agree that the KMD strategy will lead to better study design, and logically this means that "better" risk assessments take into consideration toxicokinetic data. Furthermore, if the NTP TBA chronic mouse study were conducted today using the "better" KMD approach recommended by OECD (2012), there would be no tumors to derive a slope factor, only thyroid hyperplasia for which a non-linear threshold approach to risk assessment would be applied. This is an important uncertainty with the External Review Draft derivation of a linear slope factor based on thyroid tumors.

EPA should discuss uncertainties pertaining to dose-response evaluation of thyroid follicular cell tumors (adenomas and carcinomas).

The External Review Draft appears to indicate that the toxicokinetic considerations about metabolic saturation that were raised by LyondellBasell in comments on the preliminary draft assessment were addressed in Section 2.3.2 and 2.3.4 (p. D-5 line 23-24). However, these sections do not address this and are focused on the mechanics of derivation of the slope factor.

The External Review Draft "does not agree that toxicity data collected at exposure levels which

[3] The External Review Draft incorrectly indicates that EPA (1998) carcinogenicity test guidelines states that exceeding 1000 mg/kg-d may be useful. This is from OECD (2009) but not EPA (1998) guidelines. OECD issued new guidance in 2012 updating guidance on dose selection for carcinogenicity studies. OECD (2012) repeatedly emphasizes the importance of considering toxicokinetic data and non-linear kinetics when selecting the high dose level. In addition, EPA guidance for design of chronic toxicity/carcinogenicity studies (EPA OPPTS 870.4300, 1998) defines a Limit Test as "If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effect, or if toxic effects would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary." This guidance clearly infers that if no toxicity or tumorigenicity is observed at Limit Test of 1000 mg/kg/day, and expected human exposures do not indicate they are close to the Limit Test dose, higher dose testing is not required. Higher dose testing is only required if expected human exposures are in range of the Limit Test dose of 1000 mg/kg/day.

saturate a metabolic pathway are not usable or relevant for estimating human health risk" (P. D-7, line 7-8). Even if the External Review Draft derives a slope factor based on thyroid tumors, the External Review Draft's draft assessment should include a discussion of evidence for metabolic saturation in the discussion of thyroid tumors in section 1.2.2 so that the uncertainties of the External Review Draft's cancer classification and derivation of slope factor are transparent.

The External Review Draft states that the presence of hyperplasia in all treatment groups in both sexes of mice does not support the assertion that a kinetic non-linearity exists. No MOA was identified for thyroid tumorigenesis and no mouse PBPK model is available so there is insufficient data to clearly describe the kinetics of mouse thyroid tumorigenesis (p. D-5, lines 20-38). This discussion should be included in Section 1.2.2 together with counterbalancing arguments outlined in LyondellBasell comments above that support an anti-thyroid MOA and qualitative evidence of non-linearity at the high dose group. Specifically, high-dose specific saturation of metabolic processes, including toxicokinetics, may result in transition to modes of action unique to those at high dose levels and/or represent non-dose proportional augmentation of modes of action (e.g. for thyroid hyperplasia) operating at lower animal doses.

A key consideration for TBA cancer concerns is that the genotoxicity of TBA has been extensively investigated across a variety of test systems and there is overwhelming evidence that TBA is not genotoxic.

The External Review Draft incorrectly concludes that there is a limited database available for understanding the role of TBA-induced genotoxicity for MOA and carcinogenicity, that the results are either conflicting or inconsistent, that the available data do not inform a definitive conclusion on the genotoxicity of TBA and thus the potential genotoxicity effects of TBA cannot be discounted (P. B-27). The External Review Draft's evaluation of the genotoxicity data for TBA (Section B.2.2 of the Supplemental information) does not follow EPA's or OECD's guidance on acceptable genotoxicity test battery or EPA's guidance for evaluating weight of evidence for animal studies outlined in the Preamble of this draft assessment. The External Review Draft's response to earlier comments reflects a scientific naivety and inexperience with genotoxicity data that undermines the scientific credibility of the External Review Draft assessment. The following detailed comments on genotoxicity are based on an evaluation of the External Review Draft by Dr. Bhaskar Gollapudi, an internationally recognized expert in the field of genetic toxicology.

In a series of *in vitro* and *in vivo* tests genetic toxicology tests conducted by the US NTP, it is noteworthy that TBA was consistently non-genotoxic in every one of the tests. These high quality NTP studies included 1) the bacterial reverse mutation assay, 2) in vitro chromosomal aberration test in CHO cells, 3) in vitro sister chromatid exchange test in CHO cells, 4) an *in vitro* assay for gene mutations using the mouse lymphoma cell cultures, and 5) *in vivo* mouse (two studies) as well as a rat erythrocyte micronucleus tests for clastogenicity and aneugenicity (http://tools.niehs.nih.gov/cebs3/ui/, accessed June 22, 2016). These findings from the NTP (see the table below) are in and of themselves so compelling to exclude any concern for a genotoxic or DNA-reactive MOA in the etiology of tumors observed in the long-term rodent bioassays at

relatively high doses.

In its response to the public comments, the External Review Draft states that "the two mouse studies referred to by LyondellBasell are one study published both in NTP (1995) and NTP (1997)". As listed below, these two mouse studies are different, one by drinking water (NTP Study ID: A00971; CEBS Accession No. 002-03206-0007-0000-0) and the other by 3 daily i.p. injections (NTP Study ID: A26596; CEBS Accession Number: 002-03206-0008-0000-1).

Genetic Toxicology Studies from the U.S. National Toxicology Program

No.	**Study Type**	**Endpoint Investigated**	**Result**	**Missing from EPA's assessment**
1	Bacterial Reverse Mutation Assay	Gene Mutation	Non-genotoxic	No
2	*In vitro* mouse lymphoma Cell forward mutation assay	Gene Mutation	Non-genotoxic	No
3	*In vitro* chromosomal aberration assay in CHO cells	Clastogenicity	Non-genotoxic	No
4	*In vitro* assay for sister chromatid exchanges in CHO cells	DNA breakage and reunion	Non-genotoxic	No
5	*In vivo* erythrocyte micronucleus test in male & female B6C3F1 mice (90 day drinking water study)	Clastogenicity and aneugenicity	Non-genotoxic	No
6	*In vivo* erythrocyte micronucleus test in male B6C3F1 mice (3 daily doses via i.p. injection)	Clastogenicity and aneugenicity	Non-genotoxic	Missing
7	*In vivo* erythrocyte micronucleus test in male Fischer 344 rat (3 daily doses via i.p. injection)	Clastogenicity and aneugenicity	Non-genotoxic	No

A few studies using indicator endpoints (DNA strand breakage) reported positive findings in the literature; these studies suffer from methodological issues rendering data interpretation questionable. Additionally, positive results reported in some studies could not be reproduced in independent investigations. The specific examples are listed below:

Page B-23, Line 24: The comet assay results reported by Sgambato et al. (2009) should be interpreted with extreme caution. First, the authors used a single concentration and hence the dose-response for this endpoint could not be assessed. Secondly, the single concentration used by the authors, IC50, exceeded the upper limit (IC30) recommended for in vitro comet assays (Tice et al., 2000). It is widely recognized that excessive cytotoxicity has the potential to elicit a false-positive response in this assay (Speit et al., 2015). Similarly, there was also no dose-response

information for the oxidative damage reported by these authors because of the use of a single, IC50, concentration. Without the dose-response data, it is difficult to assess the biological plausibility of the reported effects in Sgambato et al. study and this should be highlighted in the final IRIS assessment report.

Page B-24, Line 3: The discussion of Tang et al. (1997) paper should include a statement indicating that the methodology used by the authors to measure DNA damage is qualitative and highly subjective.

Page B-22, Line 14: The assessment states that "*Conflicting results have been obtained with tert-butanol-induced mutagenicity in Salmonella strain TA102, …*" This is based on the publications from Williams-Hill et al. (1999) and McGregor et al. (2005). A careful examination of these two publications reveals the following:

1. The positive response claimed by Williams-Hill et al. was very weak and did not even meet the generally accepted criterion for a positive response of 2-fold increase in revertants over the concurrent negative controls. In addition, the data presented in the publication was from a single experiment and there was no discussion on the reproducibility of this weak effect in the authors' own laboratory.
2. The publication by McGregor et al. attempted to reproduce the results of Williams-Hill et al. by testing TBA in two different laboratories and in two different solvents (water and DMSO). In none of the experiments, the revertant counts in TBA treated cultures met the criteria for a positive response (significant dose-related increase or 2 times over the concurrent negative control response).
3. In conclusion, TBA was clearly negative in TA102 in the McGregor et al investigation, even when using distilled water as a solvent to mitigate any radical scavenging influence of DMSO.

Furthermore, the External Review Draft surprisingly concludes that "*…a limited database is available for understanding the role of tert-butanol-induced genotoxicity for mode of action and carcinogenicity. The database is limited in terms of either the array of genotoxicity tests conducted or the number of studies within the same type of test*"" (line 17-19, page B-27, Supplemental Information). In its response to public comments, External Review Draft states that "Since there are a few studies that are positive, tert-butanol cannot be considered nongenotoxic with complete certainty….." The battery of genotoxicity studies conducted by the NTP includes tests for gene mutations in bacteria and mammalian cell cultures and tests chromosomal damage and aneugenicity. Gene mutations, chromosomal aberrations and aneuploidy constitute the three apical endpoints in terms of genotoxicity that are of relevance to cancer. All other endpoints, such as DNA adducts or DNA strand breaks are simply indicator endpoints that may or may not lead to apical effects and isolated positive results in non-standard assays using indicator endpoints should be given less weight in the overall weight of evidence assessment. Thus, the positive result in an in vitro comet assay reported by Sgambato et al. (2009) using a single cytotoxic concentration of TBA and the positive findings by Tang et al. (1997) in an in vitro comet assay using qualitative measures to assess DNA damage should be given less weight in the overall assessment. It is also surprising that the draft document states

that the number of studies with the same type of test is limited. Most of the studies have built-in controls to assess the reproducibility of the results and as such an adequately conducted study does not need to be repeated in multiple laboratories to arrive at a decision on the genotoxic potential for the tested substance. The WoE from a robust set of experimental studies informs that TBA does not have the potential to be an in vivo genotoxicant and thus a mutagenic MOA in the etiology of animal tumors could be excluded with a reasonable degree of scientific certainty. The agency should be able to make a similar assessment based on the existing database.

A single MOA study (Blanck et al., 2010) offers suggestive evidence of induction of Phase I and II metabolic enzymes as a postulated MOA responsible for perturbing circulating T3/T4 levels with associated centrilobular hepatocellular hypertrophy and homeostatic responses resulting in thyroid follicular cell hyperplasia and tumor induction. These data indicate that TBA is a weak CYP and SULT liver enzyme inducer in female B6C3F1 mice, sharing some PB- and CAR-like induction elements.

Taken together, the evidence suggests that TBA is not a very potent carcinogen and does not operate through a genotoxic mode of action. There is a dose dependent increase in incidence of thyroid follicular cell tumors that is not significant based on significance at the alpha level of 0.01, which is appropriate for common tumors like thyroid tumors. Although evidence of hyperplasia occurs at lower doses, the non-linear kinetics at the highest dose level and the lack of genotoxicity supports a non-linear threshold mode of action. TBA could have low potency for an antithyroid effect such that it is much more difficult to detect all the changes needed to satisfy EPA's required for this MOA compared to phenobarbital, by way of example.

4b. Cancer characterization. **As described in sections 1.2.1, 1.2.2, and 1.3.2, and in accordance with EPA's cancer guidelines (U.S. EPA, 2005), the draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for *tert*-butanol, based on thyroid follicular cell tumors in male and female B6C3F1 mice via drinking water and on renal tubule tumors in male F344 rats via drinking water. Please comment on whether this cancer descriptor is scientifically supported. If another cancer descriptor should be selected, please outline how it might be supported.**

<u>LyondellBasell Comment:</u>

Suggestive evidence is appropriate for thyroid given EPA's cancer policy and guidance for an anti-thyroid MOA. However, there is a substantial weight of evidence to suggest that the MOA for thyroid effects are not relevant to humans and occur at high dose levels not relevant for human exposures. Thus, for cancer characterization, it would be more appropriate to indicate this by providing both a threshold and non-threshold estimate for thyroid tumors to capture the uncertainty of the risk assessment.

The External Review Draft is not correct in considering kidney tumors as relevant to humans and suggesting that there is a mode of action other than CPN and α-2u-globulin for these kidney tumors (see comments to Charge question 3c for detailed discussion).

4c. Cancer toxicity values. Section 3 of EPA's cancer guidelines (2005) states: "When there is suggestive evidence, the Agency generally would not attempt a dose- response assessment, as the data generally would not support one, however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence." Please comment on whether Section 2.3 of the draft assessment adequately explains the rationale for quantitative analysis, and whether the NTP (1995) study is suitable for this purpose.

LyondellBasell Comment:

As described in detail in our response to Charge Questions 4b and 4d, several uncertainties of EPA's cancer risk assessment are not adequately addressed,

4d. Oral slope factor for cancer. Section 2.3 presents an oral slope factor of 5 x 10^{-4} per mg/kg–day, based on thyroid tumors in male or female mice via drinking water (NTP, 1995). Please comment on whether this value is scientifically supported and its derivation clearly described. If an alternative approach would be more appropriate, please outline how it might be developed.

LyondellBasell Comment:

As described in comments on Question 4a, calculation of the oral safety factor of 5 x 10^{-4} per mg/kg-day based on thyroid tumors in mice carries the additional uncertainty that the high and likely mid doses of the mouse bioassay were excessively high when considered in context of KMD and likely onset of nonlinear pharmacokinetics. Had such dose considerations been implemented in the design of the NTP mouse bioassay, thyroid tumors would not have emerged as a likely cancer concern. EPA should consider providing both a slope factor and a non-linear estimate based on thyroid hyperplasia to more fully characterize the uncertainties of the oral slope factor.

Given the External Review Draft's conclusion of an overall assessment of "suggestive evidence" of carcinogenicity, the derived safety factor should be specifically and clearly annotated with a notation that the slope factor is not suitable for use in human quantitative risk assessment and that it is only appropriate for use as a comparator to other chemicals carrying derived slope factors and/or for non-quantitative evaluations. Such a specific annotation will avoid future confusion regarding the EPA intent and scientific rationale in derivation of the TBA safety factor.

The External Review Draft also re-evaluated the slope factor derivation in male mice by excluding the high-dose response from the estimate under that assumption that excessive mortality in this dose group confounded the tumor interpretation (Section 2.3.3, p.2-20, l.22-29). Although the External Review Draft concluded that removal of the high dose group only

minimally impacted the safety factor derivation to 6 x 10^{-4} per mg/kg-day, it did not offer a comparative impact of elimination of the high dose in the female mouse thyroid tumor response. As described in comments on Section 3e, considerations that the high dose exceeded the Limit Dose and was deep into nonlinear toxicokinetic performance range offer a legitimate rationale for exclusion of this dose in the dose-response assessment of this endpoint for both male and female mice. This observation is particularly important for the female tumor response in that the overall incidence rate of thyroid adenomas in females was reported as 3, 5, 3 and 15 percent over the respective range of test doses. The female high dose tumors were the only tumor response statistically identified as significant by pair-wise comparisons (albeit to an inappropriate $p<0.05$[4]), and the significantly identified *post-hoc* trend analysis for females was likely dependent on the high-dose response given that the tumor dose-response incidence was essentially flat over the control, low and mid-dose groups. Had the high dose group been excluded in the female assessment, as was done for males, it likely would have indicated a substantive change in the derived safety factor for female mice. If this is the case, such an analysis should be considered as an additional uncertainty surrounding the lack of scientific support for use of the safety factor in quantitative human risk assessment.

4e. Inhalation unit risk for cancer. Section 2.4 presents no inhalation unit risk. The lack of a toxicokinetic model for mice precluded the use of the oral thyroid tumor data, and the inability to determine the relative contribution of α-2u-globulin nephropathy and other processes precluded the use of the oral renal tumor data from male rats. If an alternative approach would yield an inhalation unit risk estimate, please outline how it might be developed.

<u>LyondellBasell Comment:</u>

We agree that the data does not support calculation of an inhalation unit risk for cancer.

5. Susceptible Populations and Lifestages As described in Section 1.3.3, the draft assessment found inadequate information to identify susceptible populations or lifestages, due to a lack of chemical-specific data. Please comment on whether this conclusion is scientifically supported and clearly described. If there are publicly available studies to identify other susceptible populations or lifestages, please identify them and outline their impact on the conclusions.

<u>LyondellBasell Comment</u>**:**

Although there may be some limitations to the developmental neurotoxicity studies,

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[4] NTP considers a tumor response to be statistically significant at the $p<0.05$ level only when the observed tumor is a rare tumor, i.e., *less than 1% incidence* [emphasis added]. However, for tumors with background incidences greater than 1%, NTP has concluded that a p value of < 0.01 is to be used to establish statistical significance in order to avoid excessive false positive tumor evaluations (Haseman, 1983). The control thyroid tumor incidence was 5.6% in females (survival adjusted; historical control = 3.4%) and 3.6% in males (survival adjusted; historical control = 1.7%) (NTP, 1995), indicating thyroid follicular cell tumors should be evaluated to a significance standard of $p<0.01$. The pair-wise comparison of the female high dose adenoma data to controls had a p value of 0.052, while the corresponding value was 0.656 for males, both failing to meet the NTP standard of $p<0.01$. The draft assessment reported *a posteriori* significant dose response trends of $p<0.028$ for females and $p<0.041$ for males, both exceeding the statistical standard recommended for evaluations of tumors with background rats greater than 1%. Thus, using the recommended NTP statistical standards, the thyroid tumors observed in both females and males would not be considered statistically significant.

developmental studies and reproduction studies for TBA and relevant isomers; there is data that can inform the External Review Draft's evaluation of susceptible populations or lifestages for non-cancer endpoints. These studies are discussed in our response to charge question 3b.

6. Question on the Executive Summary: **The Executive Summary is intended to provide a concise synopsis of the key findings and conclusion for a broad range of audiences. Please comment on whether the executive summary clearly and appropriately presents the major conclusions of the draft assessment.**

LyondellBasell Comment:

As noted in comments above, the Executive Summary does not adequately capture:

- The key uncertainties and high degree of conservatism associated with selection of rat kidney transitional epithelial hyperplasia as the key response for the RfD derivation in that this response is a recognized element of CPN and thus not relevant to human risk;

- Uncertainties regarding the excessively high dose(s) used in the mouse thyroid tumor assessment, which exceed both the EPA and OECD test guidance for selection of a Limit Dose and other EPA and OECD guidance addressing the limitations of toxicity responses observed at dose levels saturating metabolic saturation with resulting nonlinear toxicokinetics of TBA; and

- An acknowledgment that the oral safety factor should be clearly annotated with the conclusion that the overall "suggestive evidence" of TBA carcinogenicity does not allow for its use in quantitative human risk analyses.

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